UNIVERSITY OF VERMONT

AND STATE AGRICULTURAL COLLEGE

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VERMONT AGRICULTURAL

# EXPERIMENT STATION

BURLINGTON, VT.

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## BULLETIN NO. 148

JANUARY, 1910

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## A Bacterial Soft Rot of Muskmelon, Caused by Bacillus melonis n. sp.

N. J. Giddings.

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BURLINGTON:
FREE PRESS ASSOCIATION,
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## ERRATA.

A combination of circumstances has conspired to make this issue full of errors, for none of which is the author, who had removed to another section of the country, in any way responsible. No attempt is made to correct typographical errors.

Page.

367. IV. 3. *Add* the words "Plate cultures with round colonies and saucer-shaped liquification."

368. 9. *For* starch jelly *read* silicate jelly.
*Insert*. "9 Starch jelly. Good growth in this medium containing Fermi's solution or beef broth."
*Renumber* 9-13, *reading* 10-14.

370. 16. *Insert* after words "There was good growth in broth containing" the words ".005% of formalin, fair growth in broth containing."

377. line 19. *For* tubers *read* tubes.

379. line 24. *For* recurred *read* occurred.

384. line 5. *For* in *read* a.

391. table line 4. *For* 47.4 *read* 47.9; *for* 48.9 *read* 47.9.

394. line 6. *Insert* at end the words "and from cultures 15 days old 22 c.c. of N/1 hydrochloric acid."

396. table line 2. *For* 13.2 *read* 15.2.

396. table line 7. *For* 32.3 *read* 22.3.

398. table (Glycerin) line 13. *For* 18 days *read* 15 days.

399. table line 9. *For* 15.3 *read* 15.5.
table line 13. *For* 30 *read* 20.

402. line 15. *For* .01 *read* 0.1.

403. line 18. *Insert* after ($K^2HPO^4$) the words "and .05 grams of monopotassium acid phosphate ($KH^2PO^4$) were and," eliding the verb "was."

404. *Omit sentence* beginning "These tubes."
Table line 5. *For* 3.80 *read* 3.90.

406. line 2. *For* 20-20° *read* 20-25° C.

408. line 1. *For* .05 *read* 0.5.
bottom line. *Insert* after word "of" the words "+ 5, + 10, and + 15, and fair growth in that having a reaction of."

410. line 21. *For* 48 hours *read* 24 hours.

413. line 22. *Insert* after figures 1.-1.7 the word "microns."

There are divers typographical errors in the bibliography, mostly misspelling. Such as are likely to mislead are as follows:

415. Harrison. *For* solanisaprum *read* solanisaprus.
" *For* 186 *read* 166.
Jones. *For* 229 *read* 299.
" *For* cytolic *read* cytolytic.

Figure 9 line 2. *For* ½ obj. *read* 1/12 obj.

**BULLETIN 148: A BACTERIAL SOFT ROT OF MUSKMELON CAUSED BY BACILLUS MELONIS n. sp.**

By N. J. Giddings[1]

TABLE OF CONTENTS


| | Page. |
|---|---|
| Summary | 366 |
| Occurrence and Characters of the Disease | 370 |
| Pathogenesis | 372 |
| Pathological histology | 372 |
| Inoculation experiments | 373 |
| Melon, cucumber, citron, other vegetables | 375 |
| Morphological Characters | 375 |
| Form | 376 |
| Size | 376 |
| Cultures on agar hanging blocks | 376 |
| Staining reactions | 376 |
| Flagella | 376 |
| Spores | 377 |
| Capsules | 377 |
| Involution forms | 377 |
| Cultural Characters | 377 |
| Nutrient broth | 378 |
| Agar | 378 |
| Agar stab | 378 |
| Agar stroke | 378 |
| Carbohydrate agars | 379 |
| Lactose-litmus agar | 379 |
| Agar plate cultures | 380 |
| Gelatin | 380 |
| Gelatin stab cultures | 380 |
| Gelatin plate cultures | 380 |
| Cooked potato | 380 |
| Raw vegetables | 381 |
| Muskmelon | 381 |
| Citron | 381 |
| Carrot | 381 |


[1]Formerly assistant botanist of this station; now bacteriologist of the West Virginia station.

Turnip ..... 382
Beet ..... 382
Potato ..... 382
Milk ..... 382
Viability on milk ..... 383
Litmus milk ..... 383
Fermentation broths ..... 384
Saccharose ..... 384
Lactose ..... 384
Maltose ..... 384
Dextrose ..... 385
Mannite ..... 385
Glycerin ..... 385
Urea ..... 385
Asparagin ..... 385
Blood serum ..... 386
Starch jelly ..... 386
Starch jelly in Fermi's solution ..... 386
Starch jelly in beef broth ..... 387
Silicate jelly ..... 387
Fermi's solution ..... 388
Uschinsky's solution ..... 389
Cohn's solution ..... 389
Special media ..... 389
Whey medium ..... 389
Curd medium ..... 389
Curd medium with lactose ..... 389
Casein medium with mannite ..... 389
Casein medium ..... 390
Physical and Biochemical Characters ..... 390
Temperature relations ..... 390
Maximum temperature ..... 390
Minimum temperature ..... 390
Growth at different temperatures ..... 390
Thermal death point ..... 391
Growth in carbon dioxid ..... 391

Indol production ..... 392
Nitrite production ..... 393
Ammonia production ..... 393
Acid production ..... 394
Titration tables ..... 395
Gas production ..... 400
Comparison with B. coli ..... 400
Gas from milk ..... 400
Special media ..... 402
Whey medium ..... 402
Curd medium ..... 402
Curd medium with lactose ..... 403
Casein medium with mannite ..... 403
Casein medium ..... 403
Milk analyses ..... 404
Alcohol production ..... 405
Hydrogen sulphid production ..... 407
Odor ..... 407
Growth over chloroform ..... 408
Tolerations ..... 408
Oxalic acid ..... 408
Malic acid ..... 408
Sodium chlorid ..... 408
Hydrochloric acid ..... 408
Sodium hydroxid ..... 409
Effect of germicides ..... 410
Phenol ..... 410
Formalin ..... 410
Desiccation ..... 411
Insolation ..... 411
Remedial Measures ..... 412
Brief Description of *Bacillus Melonis n. sp.* ..... 413
Bibliography ..... 415

Since this bulletin is of a technical nature and will be used primarily by bacteriologists for reference, the following brief summary is inserted before the body of the article.

## SUMMARY

I. *Occurrence and character of the disease:*

A soft rot of the muskmelon caused by a bacillus. The character of the decay is similar to that of other vegetable soft rots, but the organism differs from the soft rot type, *Bacillus carotovorus* (Jones).

II. *Pathogenesis:*

1. Pathological histology.—The tissues very rapidly disorganized and cells dissociated, due to the enzymic softening of the middle lamella. Material imbedded, sectioned and stained, showed bacteria working along the plane of middle lamella.

2. Inoculation experiments.—Wound inoculations in muskmelon generally gave complete decay in from three to seven days. Wound inoculations in citron and cucumber fruits resulted in decay. Raw sterile blocks of muskmelon and citron fruits, carrot, turnip and beet roots and potato tuber were decayed. Portions of the tissue of carrot, turnip, and potato were considerably discolored. No decay was secured in inoculated wounds in fruits of squash or summer squash. Inoculations of muskmelon upon unbroken surface did not bring about decay.

III. *Morphological characters:*

1. Form.—A bacillus; short rods with rounded ends occurring most often singly, but frequently in chains of two or three.

2. Size.—Stained specimens of young cultures averaging about .7 micron by 1.2 microns; unstained specimens about .9 micron by 1.6 microns.

3. Agar hanging block.—Organisms generally occur as single rods, sometimes in chains of two or three, very rarely in chains of four to five. The orientation of chains was irregular.

4. Endospores.—None were found.

5. Motility.—Very actively motile in decaying tissues and young liquid cultures. Four to six peritrichic flagella readily stained by Loewit's method.

6. Capsules.—None observed.

7. Involution forms.—None of importance noted.

8. Staining reactions.—Young organisms readily stained with aqueous solutions of fuchsin, gentian violet or carbol fuchsin. Not stained by Gram's method.

IV. *Cultural characters:*

1. Nutrient broth.—Strong transient clouding and slight sediment. No pellicle or ring formation.

2. Agar.—Filiform growth entire depth of stab. Slightly spreading with umbilicate elevation, slightly contoured. Surface colonies round to amoeboid. Buried colonies spindle shape. No fermentation in carbohydrate agars. Acid production in lactose-litmus agar.

3. Gelatin.—Liquefaction apparent in 24 hours, at 20° C., complete in 14 days. Stab cultures with infundibuliform liquefaction.

4. Cooked potato.—Growth abundant, spreading, slightly contoured, glistening, and with a decided odor of decaying potatoes.

5. Raw vegetables.—Good growth on sterile blocks of muskmelon, citron, carrot, turnip, beet and potato. Discoloration of potato, beet, turnip and carrot.

6. Milk.—Coagulated in 24 to 48 hours at 30° C. No digestion. Cultures in milk were alive at the end of 36 days. Litmus milk coagulated and reddened in 3 days. Litmus completely reduced in 30 days. Return of color in 40 to 60 days.

7. Fermentation broths.—Good growth in saccharose, lactose, maltose, dextose, mannite, glycerin, and asparagin; slight growth in urea; good growth in closed arm in mannite, saccharose and asparagin; no growth in closed arm in glycerin.

8. Blood serum.—Growth effuse, glistening and slimy with echinulate edges. Substratum became clear in 10 to 15 days. Slow but distinct liquefaction.

9. Starch jelly.—Very abundant growth. Inclined to be spreading with echinulate edges, slightly contoured, umbilicate elevation, and slimy. Slight greenish yellow discoloration of substratum.

10. Fermi's solution.—Strong, persistent clouding with ring formation, and considerable sediment.

11. Uschinsky's solution.—Very strong growth in this medium, with ring and pellicle formation and large amount of sediment. Disagreeable odor of hydrogen sulphid.

12. Cohn's solution.—No growth.

13. Special media.—Good growth in whey medium, curd medium, curd medium with lactose, and casein medium with mannite; slight growth in casein medium. Coagulation in casein with mannite.

V. *Physical and biochemical characters:*

Maximum temperature of growth 40° C.; [1]minimum temperature, 0° to 1° C.

1. Temperature relations.—Optimum temperature for growth about 30° C.; slight growth at 37.5° C; thermal death point 49 to 50° C.

2. Growth in carbon dioxide.—There was very slight growth in gelatin stab and agar stab cultures.

3. Indol production.—A slight reaction was secured in 10 days; more pronounced reaction in 20 days.

4. Nitrite production.—Abundant in 5 days.

5. Ammonia production.—Abundant from fermentation tube cultures of asparagin broth; none in broth, gelatin, milk or urea.

6. Acid production.—Slight in mannite, urea, glycerine, dextrose, lactose, saccharose, and maltose; pronounced in milk.

7. Gas production.—No gas in fermentation tubes of lactose, maltose, dextose, saccharose, glycerin, or mannite; slight gas production in fermentation tubes of asparagin broth; abundant in fermentation tubes of milk, this gas being at least 99% carbon dioxid. A whey medium, secured by coagulating milk with rennet, gave 30 to 54% gas, of which 99% was carbon dioxid. No gas was produced in some special casein media which were tried as substitutes for milk. Analyses of fermented milk showed a remarkable diminution in lactose content with a slight increase in acid content, which was not, however, sufficient to account for the loss in lactose. It is thought, therefore, that some lactose was used up in the production of carbon dioxid. Distillation of inoculated milk at the end of 15 days gave some evidence of alcohol.

8. Hydrogen sulphid production.—This was found in cultures on nutrient broth and cooked potato.

9. Odor.—No very distinctive odor was noted.

10. Growth over chloroform.—A good growth in 24 to 48 hours in tubes containing 5% of chloroform.

11. Toleration of sodium chlorid.—Good growth in nutrient broth containing 1, 2, 3, 4, 5, and 6% additions; a fair growth in those containing 7%; and slight growth in those containing 8%. Organisms were killed in 10 days in tubes containing 9 or 10%.

12. Toleration of hydrochloric acid: There was good growth in broth having reactions of +5, +10, +15, and +20; a slight growth in broth having reaction of +25; and no growth in media having a higher reaction.

13. [1]Toleration of oxalic acid.—The organism grew in a beef bouillon acidified to +45 and +47 with oxalic acid, but did not grow in one acidified to +52.

14. [1]Toleration of malic acid.—Growth was secured in beef bouillon acidified to +37 with malic acid.

15. Toleration of sodium hydroxid.—Good growth was secured in broths incubated in carbon dioxid free atmosphere

and having reactions of —5 and —7.4. Growth was not secured in such tubes having reaction of —10.2.

16. Effect of germicides.—Good growth was secured in broth containing .02% of phenol crystals; fair growth in broths containing .05% and .08%; very slight growth in broths containing .09% and .11%; no growth in the presence of .18% or more of phenol. There was good growth in broth containing .01% very slight growth in broths containing .02% to .04%; none in the presence of .05% or more.

17. Desiccation.—Organisms were killed by drying for 7 days, and vitality was greatly reduced after drying for 68 hours.

18. Insolation.—Exposures of 15 minutes killed 80% of the colonies, while an exposure of an hour killed all the colonies.

VI. *Remedial measures:*

Spraying with bordeaux mixture or other fungicides recommended as a preventive measure, coupled with lifting and turning of the melons, and irrigation if needed. Diseased melons should be immediately destroyed. Rotation of crops should be practised in a field where the disease has occurred.

## OCCURRENCE AND CHARACTERS OF THE DISEASE

During September, 1907, Mr. E. S. Brigham, of St. Albans, suffered a considerable loss in a field of Montreal muskmelons which he was growing for fancy market. This crop was in

[1]Dr. E. F. Smith of the Bureau of Plant Industry of the U. S. Department of Agriculture, kindly contributed confirmatory and additional observations at several points. He determined the liquefaction of Loeffler's blood serum, the maximum and minimum temperatures in peptonized beef bouillon, and the toleration of the organism for oxalic and malic acids; and he advised the writer as to certain points concerning the growth of the organism on Fermi's solution.

a measure an experimental one, being grown in cooperation with the horticultural department of the Station, to determine whether such a crop would prove profitable in this state.[1] The plants were grown in frames, sash being kept over them until the latter part of August. The season was very dry until September, and as a result the growth of the melons had been somewhat slow. During early September, however, there were heavy rains, and these, combined with warm cloudy weather, induced a very sudden increase in growth, which resulted in numerous cracks in the skin and flesh. These cracks furnished excellent points of entry for pathogenic organisms, and the succulent tissue evidently furnished a favorable medium for their growth. As a result the melons were attacked in early September by a soft rot, which destroyed about twenty-five per cent of the crop before the end of the season. The decay generally began on the under side of the fruit and spread rapidly. The skin became somewhat shrunken over the diseased area but generally remained unbroken. When the melon had entirely collapsed there was usually some frothing and a very disagreeable odor. Some of the melons which became diseased lay in natural contact with the ground, but others were fruits which had been placed on blocks.

Specimens of the diseased melons were first brought to the writer on September 14, by the station horticulturist. Microscopic examination was made at once. It was found that the decaying tissues swarmed with motile bacteria, and that the cells were dissociated. A large number of agar plates were poured from one of these melons which was in the early stages of decay. Numerous bacterial colonies appeared on these plates within less than 30 hours. The colonies were practically all similar in appearance and broth transfers and inoculations were

[1]See for detailed account of the experiment and conclusions, Stuart, Wm., The Market Muskmelon Industry, Vt. Sta. Rpt. 20, pp. 358-366 (1907). Considerable of the field data here given is taken from this article.

made from some of them at this time. Transfers were made first so as to secure the organism in pure culture, and then inoculation was made from the same colony. In 48 hours the decay was well under way in melons thus inoculated. It seemed evident that the decay was caused by one organism and all further cultures were taken from these similar colonies. Some further inoculation experiments were made and the organism was retained in pure culture, in order that a detailed study of its characters might be made.

## PATHOGENESIS

### PATHOLOGICAL HISTOLOGY

A microscopic examination of the diseased tissues from inoculated muskmelons gave evidence that the organism had invaded the intercellular spaces, dissolving the middle lamella so that the cells were frequently seen floating around loose. In many cases also the bacteria were seen to have fully invaded the spaces between the cells. The remaining part of the cell walls appeared swollen, but bacteria were not observed in the interior of cells which had recently been separated from the others through the disorganization of the middle lamella. For more critical examination, sterile blocks of raw muskmelon were cut and dropped into young broth cultures of the organism, in which they were allowed to remain for 15 to 20 hours. These pieces were then cut into smaller blocks and killed and fixed in boiling absolute alcohol. They were imbedded in paraffin, sections cut, and various staining reagents tried, in order to bring out the histological relations of the organisms and tissue. This method confirmed the conclusions stated above, that the invasion is strictly intercellular and that the principal effect on the wall is the solution of the middle lamella. Good staining reactions were secured with Zeihl's carbo-fuchsin, bismarck brown, and Loeffler's alkaline methyl blue. A differential stain was secured by using bismarck brown for two minutes and the methyl blue

about twenty seconds. The action of the organisms upon the middle lamella appears similar to that of the carrot rot organism, *Bacillus carotovorus,* Jones.

### Inoculation experiments

All the vegetables used for this purpose were carefully cleaned and washed previous to inoculation, but were neither soaked in nor washed with corrosive sublimate. Such as were inoculated in the laboratory were wrapped with moist absorbent paper and placed in moist chambers at 20° to 27° C.

*Melon:* Melon No. 1. This specimen, about three fourths grown, was secured from a local market gardener. It was inoculated on Sept. 18 from an agar culture 24 to 48 hours old, using a 2 mm. loop to transfer some of the condensation water to a small skin wound made with a sterile scalpel. In 24 hours a watery spot about one-half inch in diameter appeared around the point of inoculation. In 48 hours this spot was about one inch in diameter, and a little water oozed from the wound. On opening the melon tissue was found to be somewhat softened in an irregular funnel shaped section extending from the surface into the cavity, its diameter at the point where it entered the cavity being about one sixth of an inch.

Melon No. 2. A full grown, unripe, nutmeg was secured from a local gardener. A small wound was made in the surface with a sterile, platinum-iridium needle. The inoculation was made from a 48 hour old agar culture, in the same manner as with No. 1. In 24 hours a softened area of one-half inch in diameter had developed around the point of inoculation, which in 48 hours was two and one-half inches in diameter, the skin over the softened portion having sunken until nearly flat. At the end of 72 hours one half of the melon had fallen in and the next day it was entirely softened.

Melon No. 3. A nutmeg, handled in the same manner as No. 2. At the end of 24 hours the softened area was about one-half inch in diameter, in 48 hours it was three inches, and at

the end of the third day the melon had fallen in and, save a portion of firm flesh on one side, was entirely softened.

Melon No. 4. A cantaloupe, handled in the same manner as No. 2. In 24 hours the softened area was about one-half inch, and in 48 hours about three inches in diameter. In 72 hours it had entirely decayed and showed slight frothing.

Melon No. 5. A nearly full grown nutmeg, inoculated on the vine. Inoculation was made as with melon No. 2 and several thicknesses of moist filter paper were wrapped around it. In 48 hours a softened area about one-fourth inch in diameter had developed, which at the end of three days was about one-half inch, and in five days about three-fourths of an inch in diameter. In ten days the melon had fallen in. The weather was quite cool when the field inoculations were made, which may account for the slow progress of the decay.

About twenty melons were inoculated during the fall of 1907, and about a dozen during the next fall; four of these while on the vines and the remainder in the laboratory. Decay followed every wound inoculation with results essentially like those described above. A number of these melons were opened at different stages of decay in order to note the mode of progress. The organisms appeared to work in from the surface, spreading toward the center and widening out under the skin at nearly the same rate, so that a funnel shaped area was first softened, which finally penetrated into the cavity. As soon as the organisms entered the cavity they spread very rapidly over the entire interior surface and worked from this toward the skin. Whenever more than one melon was inoculated, and in some cases when only one was under trial, controls were made by wounding another part of the same melon, or other melons, as though to make inoculations. These were in all cases treated in the same way as were the inoculated wounds, save that no organisms were placed in them. Rot developed in none of these control wounds. Furthermore, two healthy, uninjured melons while growing on the vines were sprayed with portions of broth

culture of the organism, 24 to 48 hours old, but no signs of decay occurred within seven days.

*Cucumber.* Nine cucumbers, varying in age from those quite young to such as were full grown but not ripe, were inoculated in the same manner as were the muskmelons. These showed no signs of decay, other than a very small spot, until four days after inoculation. The invasion by the organism then became evident in the younger cucumbers, and in seven days after inoculation four of the fruit were entirely collapsed. Three of the others showed no signs of decay, while the two remaining, which were opened at this time, revealed a small decaying area around the point of inoculation. The organism is evidently capable of decaying cucumbers, but with much more difficulty than in the case of muskmelons.

*Citron.* Specimens of citron fruit were secured in the market, cut with sterile knives, and inoculated on the freshly cut surfaces, taking material from a 24 to 48 hour old broth culture, using a 2mm. oese. The organisms spread very rapidly in this tissue, the citrons being decayed on the fourth or fifth day.

*Other vegetables.* Several fruits of squash and summer squash were inoculated, but no decay ensued. Sterile blocks cut from the roots of raw carrot, turnip and beet, and from raw potato tubers were quite rapidly rotted, as will be described under cultural characters.

These results indicate that the organism in question is different from *Bacillus carotovorus,* Jones, since that organism was found in no case to cause decay of the beet. Moreover its invasion of the potato tuber was less active than that of this melon-rot organism.

## Morphological Characters

The morphological characters of the organism were studied in broth and agar cultures 24 to 48 hours old, with the exception of the work done in searching for spores.

*Form.* This organism is a bacillus having rounded ends and occurring generally as single cells, frequently in pairs, and occasionally in chains of three or, rarely, of four or five. The grouping in both agar and broth cultures was very similar.

*Size.* Stained specimens from cultures about 36 hours old, incubated at 25° C., using gentian violet as a stain and taking measurements with an ocular micrometer, afforded dimensions of .5 to .8 by .9 to 1.5 microns. To secure measurements of greater exactness, photomicrographs of the living organism on agar hanging block cultures, and of the divisions on a stage micrometer, were taken under identical conditions. The results indicated that the living organism in agar hanging block cultures possessed a diameter of .8 to 1. micron and a length of 1.5 to 2. microns. (See figure 9).

*Cultures on agar hanging blocks.* The agar hanging block cultures were made by taking a transfer from a young broth culture and smearing it over the surface of a small block of sterile agar. A cover slip was then placed upon the block with the inoculated side next to the glass. These were allowed to remain at room temperature, 22 to 25° C, and the development of the organisms was observed. There appeared to be no special grouping of the organisms other than has already been noted above under "Form."

*Staining reactions.* The organism was well stained by a watery solution of gentian violet, watery solution of fuchsin, and carbol-fuchsin. The latter, applied cold, gave a deep color in 30 seconds; the others required about one and one-half minutes. The organisms from young cultures appeared to be strongly plasmolyzed by carbol-fuchsin, giving what might be called a polar stain, the ends of the rods being generally stained as though the protoplasm had left the middle portions and gathered at the ends. The organisms did not stain by Gram's method.

*Flagella.* The organisms were actively motile in beef broth, Fermi's solution, Uschinsky's solution, and other liquid

media incubated at 20°, 25°, or 30° C. They were also very actively motile in freshly invaded muskmelon tissues. The movements were frequently darting. They were found to be motile by means of 4 to 6 peritrichic flagella. Cultures in a medium composed of one drop of Uschinsky's solution to seven c.c. of distilled water and incubated for 18 hours at 25° C. gave the best material for staining. Material transferred directly from this to clean cover slips and stained by Loewit's method showed excellent flagella. Good material for staining was also secured by using the condensation water from agar stroke cultures 24 to 36 hours old, making a dilution thereof in sterile distilled water. Fair results were secured by staining according to Pitfield's method, and less satisfactory results, showing a few flagella poorly stained, by VanErmengem's method.

*Spores.* In no case has anything resembling spores been seen, nor has there been any indirect evidence of spore formation. Transfers were made to broth from nutrient agar cultures and from potato cultures 30 to 60 days old. These inoculated tubers were then placed at once in a water bath at 65° C., and were in all cases killed by a 10 minute exposure.

*Capsules.* Capsule formation was noted in no case.

*Involution forms.* No involution forms worthy of note were observed.

## Cultural Characters

The culture media used were carefully prepared following directions as given by Smith[1] and the publications of the American Public Health Association.[2]

Once distilled water was used in preparing all media, unless otherwise stated. The formula used for making nutrient broth was Witte's peptone, 10 grams, Liebig's extract of beef, 5 grams,

[1]Smith, E. F., Bacteria in Relation to Plant Diseases, Vol. I, (1905).

[2]Report of Committee on standard methods of Water Analysis, American Public Health Association.

C. P. sodium chlorid, 5 grams, distilled water, 1 litre. The reaction of all media containing nutrient broth was plus 10, Fuller's scale, unless otherwise noted, the titration being made with N/20 sodium hydroxid, using phenolphthalein as an indicator. For all cultures, unless otherwise specified, culture tubes about 13. by 1.5 cm. in size were used containing 7 c. c. of the medium. All transfers, except those for determining spore production, were made from one to four day old broth cultures. Transfers to fluid media were made with a 2 mm. loop, and those to solid media were made with a straight needle.

### NUTRIENT BROTH

The growth in nutrient broth was rapid with strong clouding. No pellicle or ring formation was noted. At 30° C. it was very strong in 24 hours and did not increase perceptibly after 48 hours. The medium became clear in about two months. A slight amount of sediment formed which showed but slight increase at the end of a month.

### AGAR

All agar media contained 1.5% of agar flour.

*Agar stab* cultures showed a medium filiform growth along their entire depth. There was a slight but not well marked tendency in some tubes toward a villous or papillate growth along the line of puncture. The surface growth was abundant and spreading, frequently extending to the tube walls if the medium had been melted just before inoculation and was in consequence moist. There was no sign of liquefaction or gas formation in any agar tube.

*Agar stroke* showed good growth within 24 hours, which was slightly spreading and filiform with wavy margins. The elevation was umbilicate, the surface slightly contoured, and the growth transluscent with a slight opalescence. It was glistening and slimy, without color, and did not discolor the medium. (See figure 12).

*Carbohydrate agars.* These were made up containing respectively 5% saccharose, dextrose, lactose, mannite and glycerin. Other series contained 2% of all these except glycerin. Shaken cultures were made in each of the above agars and in ordinary nutrient agar. A control culture of *Bacillus coli* was also made on each of these agars. The melon rot organism showed good growth at or near the surface in all tubes in 24 hours, the best growth being secured upon 5% glycerin and the least upon 5% saccharose, 5% dextrose, and upon plain nutrient agar. The buried colonies were apparent in 2 days and at the end of the week the medium was strongly clouded by their great numbers, which were much more numerous toward the surface then deeper. The tubes of *B. coli* showed well marked gas production in each medium save the plain agar, but no sign of gas production appeared in any tube inoculated with the melon rot organism.

*Lactose-litmus agar.* Agar containing 2% lactose was used in this series of cultures. Series (A) was prepared by adding to this 2% of a solution of one part of C. P. litmus in fifteen part of water, and series (B) by adding 5% of neutral litmus solution.[1] Both shaken and stroke cultures were made upon each of these media. Within 24 hours a slight acidity developed in the shaken tubes of A, and in 48 hours marked acid production had recurred in both shaken and stroke cultures of both A and B. These remained unchanged except for a slight deepening of the red color until the end of a week, when slight bleaching in the stroke cultures of B was observed. In ten days the shaken cultures of B were entirely bleached except a very little near the surface, and the shaken cultures of A showed slight bleaching. At the end of 15 days the A tubes were about one-half bleached, and B tubes entirely bleached. No gas formed in any tube. The C. P. litmus gave the better

[1]See Prescott and Winslow, "Elements of Water Bacteriology," p. 126. (1904.)

results with both these organisms. It indicated acid production by a bright cherry red, whereas the other medium only showed a dull red tint.

*Agar plate cultures.* The growth was very rapid at from 25 to 30° C., forming colonies over 1 mm. in diameter in 18 hours at 25° C. In general the colonies were round but occasionally became amoeboid, the surface was smooth with very slight convex elevation, the edge entire and the internal structure finely granular. The colonies were slightly opalescent with moist, glistening surface. Buried colonies were gradually spindle shaped but occasionally elliptical.

GELATIN

*Gelatin stab cultures* showed a slight liquefaction after 24 hours at 20° C. The growth along the line of puncture was filiform in 18 to 24 hours, but liquefaction began almost at once so that it was hard to secure any other growth characters. At the end of a week the gelatin was about 80 per cent or more liquefied, and there was a large amount of flocculent sediment. Complete liquefaction resulted in 14 days. Gelatin tubes inoculated and placed at 25° C. were about one-third liquefied in 24 hours, and in 48 hours were entirely liquefied. The liquefaction in all stages and at both temperatures was infundibuliform to saccate. (See figure 12).

*Gelatin plate cultures* at 20° C. showed liquefaction within 40 hours. The colonies at first were round, becoming in about 36 hours reticulate; then after 4 to 8 hours liquefaction began and the colonies again became round, the edges after this stage being floccose. The colonies remained round and the liquefaction was saucer-shaped.

COOKED POTATO

The growth was light yellow, spreading, abundant, slightly contoured and glistening. There was good growth in 48 hours. The growth was slimy and the tubes had a decided odor of de-

caying potatoes. There was no discoloration. In 15 days the growth in cultures at 25° and 30° C. was much less conspicuous than at first. At 37° C. there was no visible growth.

### RAW VEGETABLES

Sterile blocks of muskmelon, citron, potato, carrot, beet and turnip were secured in the following manner: The room which was to be used for cutting the blocks was carefully sprayed with water, wetting down the walls and sending a fine spray throughout the air of the room. The vegetable used was first carefully washed and then either soaked or washed for 3 to 7 minutes in a 1-1000 solution of mercuric chlorid. The outer portions were then removed with hot sterile knives, sterile blocks cut from the interior in the same manner, and these blocks were placed in test tubes containing 1-2 c.c. of sterile distilled water. Before inoculation the tubes so prepared were in all cases incubated for 2 days at 25° C. and any tube which did not appear sterile at the end of that time was discarded. Out of nearly 300 blocks cut in this manner there were only some half dozen which did not appear to be sterile after such trial incubation.

*Muskmelon.* Blocks of raw muskmelon were entirely collapsed in two days. The blocks had a watery appearance at the end of 20 to 24 hours, and soon sank down into the water forming a semi-fluid mass. There were some indications of gas production.

*Citron.* Inoculated blocks of this vegetable showed much the same appearance as did the blocks of muskmelon, but frequently did not collapse until the third day after inoculation. There was no discoloration other than the watery appearance observed in the muskmelon. Slight gas production occurred.

*Carrot.* Inoculated carrot blocks were entirely softened in three to five days. These did not collapse spontaneously as did the muskmelon and citron, but upon shaking would do so. The

portion of the block above the water was darkened, frequently becoming almost black. Slight gas production occurred.

*Turnip.* Blocks of this vegetable were discolored to the extent in some cases of turning nearly black and frequently in cases they showed a somewhat mottled appearance. The blocks were usually considerably softened in three to five days, but less so than the carrot blocks; and they did not collapse as readily. In some cases, where there was insufficient water in the tube to keep the surface of the blocks moist, growth was retarded or fully checked and little or no decay resulted.

*Beet.* The immersed portions were completely softened within three to five days and entirely so within a week. They did not collapse however, even upon shaking, but a platinum needle could readily be passed through them anywhere. The non-submerged portions were nearly black. Slight gas production occurred. In case of insufficient moisture the growth was retarded or even completely checked in the same manner as occurred when the turnip was used.

*Potato.* Blocks of potato were nearly one-half softened within three days. If pressed with a platinum-iridium needle the submerged portion would entirely collapse and spread around as a thick mass. These blocks were entirely softened within a week, in case there was enough water in the tubes to prevent them from drying out. The portions above the water were nearly black. Some gas production occurred in these tubes.

### MILK

Fresh, centrifuged milk was filtered through two thicknesses of Schleicher and Schull filter paper and 10 c.c. used in each tube. It had a reaction of between +10 and +15 Fuller's scale. A small coagulum formed in 24 hours after inoculation at 30° C. This was not visible on looking at the tube, but when the milk was poured out it appeared as a small soft lump. In

48 hours the milk was entirely coagulated and showed some signs of separation; in four days this was quite complete, but the curd continued to shrink for several days longer. The action of the milk was slower at 20° or 37° C., coagulation at either temperature not occurring until the fourth day. Such tubes at 37° as were not coagulated within four days time did not coagulate later. After five to seven days at 20° and 25° C. small bubbles of gas were evolved when the tubes were disturbed. No evidence of curd digestion was seen.

*Viability in milk.* In order to test the duration of viability, transfers to tubes of sterile broth were made from cultures on milk at the end of each 10 days, 15 days, 22 days, and 36 days. Growth was secured in every case. All transfers from such tubes which had been incubated during this period showed strong clouding after 24 hours at 25° C., while those from tubes at 37° C. showed only slight clouding after 24 hours. (For acid and gas production in milk see later discussion of biochemical characters, pages 394 and 400).

*Litmus milk.* Tubes of litmus milk were made up by adding to ordinary milk, when ready for tubing, 0.1% azolitmin. Tubes of this milk were not entirely coagulated until three days after inoculation, showing a slight retardation as a result of the addition. Acid production was evident two days after inoculation. This became more marked until the fifth day, and at this time reduction of the litmus began. This proceeded rather slowly until it was complete in 20 to 30 days. Tubes at the higher temperature of 30° C. showed a return of the neutral lavender color in 40 to 60 days. Inoculated tubes of this litmus milk and of ordinary milk were titred at the end of 45 days and found to have a reaction of +75 to +85 Fuller's scale, showing that the return of color was not associated with any decrease in acid content. Otherwise these tubes behaved in the same manner as did the tubes of ordinary milk.

FERMENTATION BROTHS

Using the standard beef as a base, fermentation broths were prepared containing 2% additions respectively of saccharose, lactose, maltose, dextrose, mannite, glycerin, urea and asparagin. These media were put up in uniform fermentation tubes having a height of about 15.5 cm. in diameter of 1.3 cm. and a well marked constriction between the open and closed arms. Twenty c.c. of broth was placed in each tube. The work with all of the broths, except urea, was repeated three times, using eight tubes of the same medium in each series; that with the urea was repeated only twice. Inoculations in these tubes were made simply in the surface of the open arm. These trials indicated the organism to be a faculative anaerobe, since the stronger growth always occurred in the open arm, but in practically every case some growth was made in the closed arm. In no case did any gas form. The details of the trials follow:

*Saccharose.* Growth at 30° C. was very good in the open arm in 24 hours, with slight growth in the closed arm. At from 48 hours to five days there was marked difference in the growths in the open and closed arm, the growth being much the stronger in the former. This difference remained more or less apparent in cultures as old as 20 days, but growth in the closed arm was quite good in five days. In a week there was a small amount of granular sediment, which continued to increase until the tubes were 20 days old, when the closed arm had become almost clear. At 37° C. a fair clouding occurred in both the open and closed arms within 24 hours, the latter becoming almost clear in five to seven days, with a slight amount of sediment.

*Lactose.* The growth was very similar to that in saccharose, but at 30° C. the difference between open and closed arm was more marked. The growth was somewhat stronger, and more sediment formed.

*Maltose.* The growth was similar to that upon saccharose but the difference in growth in the closed and open arms remained quite marked in tubes 20 days old.

*Dextrose.* The growth was very similar to that on saccharose but with less marked difference between the growth in the open and closed arms.

*Mannite.* A much stronger growth in the closed arm was observed than in any of the preceding broths. There was, however, in cultures two to three days old but slight difference between the open and closed arm. Clearing in the closed arm at 30° C. began in about eight days and was almost finished in fifteen days. Tubes at 37° C. were almost entirely clear after five days, but a small amount of sediment remaining.

*Glycerin.* A strong growth occurred in the open arm, but the closed arm remained perfectly clear in almost every case. Where slight clouding in the closed arm did occur, it seemed due to the slight circulation of the broth caused by handling the tubes. The growth in the open arm and connecting tube was so dense as to give the broth a milky appearance. At 30° C. the tubes had only begun to show signs of clearing in 15 days, while at 37° they were nearly cleared in 10 days.

*Urea.* This was a very unsatisfactory medium, for upon sterilization by flowing steam a clouding and slight precipitation ensued, doubtless indicating some change in the medium. In no case was a good growth secured. It was slight and, because of the natural clouding, it was uncertain whether growth occurred in the closed arm or not. It ensued in the open arm, and a line showed the difference in clouding between the open arm and the closed arm. The broth was almost clear in 10 days at 30° C., and a small amount of sediment developed.

*Asparagin.* The growth was good, and fairly well marked differences were observed between the growth in the open and the closed arms. The clouding developed in the closed arm which was very pronounced in three to eight days at 30° C., though possibly not quite so strong as in mannite. The growth in the open arm was stronger than in any of the other broths, save perhaps glycerin. A large amount of flocculent sediment

formed at the base of the closed arm and in the constriction leading into the open arm in about five days. This did not increase much, but its texture became finer in about seven days, forming a somewhat compact mass. The closed arm began to clear in 7 to 8 days at 30° C., and in 15 days was almost entirely clear.

### BLOOD SERUM

Stroke cultures on this medium showed good growth in 24 hours at 30° C. It was effuse with a slight tendency to be umbilicate in elevation. The edges were strongly echinulate, the surface slightly contoured, and the growth glistening and slimy. A very pronounced milky clouding occurred in the condensation water, but there was no increase in its amount as the growth progressed. The substratum, which was quite cloudy at first, became clear in cultures ten to fifteen days old. There was slow but distinct liquefaction.

### STARCH JELLY

Starch jelly was made as follows: 7 c.c tubes of the fluid medium used were heated to from 65° to 70° C., and to each was added .7 grams of pure potato starch and the mixture thoroughly stirred with a glass rod. The tubes were then immediately placed slanting in a water bath at 80° to 90° C.[1] The tubes were then sterilized by heating for two hours on each of 6 consecutive days at 80° to 90° C., in a water bath in which beakers of water were placed to maintain a moist atmosphere.

*Starch jelly in Fermi's solution.* The growth was very good. It was a trifle too wide for a typical filiform growth,

[1]Careful attention to the temperature is essential to success in making this medium. If the temperature of the liquid is too high when the starch is added, swelling begins so promptly that the starch forms a thick mass, which clings to the stirring rod and cannot be properly mixed with the liquid. If, on the other hand, the temperature is too low, the starch settles before swelling begins. When the temperature is properly regulated a homogeneous jelly is secured.

had echinulate edges, umbilicate elevation, was more or less contoured, glistening, very slightly viscid, light yellow, and did not cloud or discolor the medium.

*Starch jelly in beef broth.* Cultures showed a good growth but not as strong a one as that which formed upon the starch jelly in Fermi's solution. There was no viscidity, the color was almost white, and no clouding or discoloration of the medium was observed.

### SILICATE JELLY

This medium was prepared as follows: C. P. hydrochloric acid was diluted with distilled water to a specific gravity of 1.1 at 15.5° C. A solution of sodium silicate was diluted with distilled water to a specific gravity of 1.085 at 15.5° C. Collodion sacks were made by pouring a 5% solution of collodion into a large test tube, (size about 3x25 cm.), rotating the tube meantime, and, when sufficient had been added so that there was about 2 or 3 cm. of fluid in the bottom of the tube, at once pouring this out, rotating the tube constantly. It was then allowed to drain bottom up for two to three minutes, the excess collodion wiped from the lip of the tube, and the tube rotated in front of an electric fan for 5 to 8 minutes. The tubes were then filled with water, the top portion of the collodion sack separated from the test tube, and the entire sack carefully withdrawn. These were then placed in distilled water until wanted. Equal parts of the sodium silicate and hydrochloric acid were measured out, and the sodium silicate added by permitting it to drop rapidly into the acid, stirring thoroughly meanwhile. This solution was then poured into collodion sacks, each sack receiving about 70 c.c. The mouths of the sacks were fastened firmly with rubber bands, they were immersed in running water and permitted to dialyze for 12 hours. They were then taken out, the silicate jelly poured into a flask and boiled for two or three minutes. In the meantime some Fermi's solution was

made up, using only one-tenth as much water as the formula calls for. The air was exhausted from this solution in a vacuum chamber, the silicate jelly was cooled down to about 40° C. and the Fermi's solution added. It was then carefully mixed, tubed, slanted and at once sterilized under 8 pounds of steam pressure.

The growth upon this medium was very abundant in 48 hours. It was filiform but somewhat inclined to be spreading, with echinulate edges, glistening, slightly contoured, umbilicate in elevation and slimy. The condensation water was very milky. The growth was grayish white, translucent-opalescent, and after 5 days it showed a slight viscosity and a very slight greenish-yellow discoloration of that portion of the medium forming the slant. In about 8 days small rib-like out-growths appeared on the under surface of the stroke, which in 10 to 15 days frequently appeared to have formed on the mid-rib a tooth-like growth. (See figure 13). The medium was somewhat inclined to split, and wherever such splitting occurred the organism quickly appeared as a heavy growth. The amount of growth continued to increase visibly until the cultures were at least a week old and was more abundant upon this than upon any other solid medium.

### FERMI'S SOLUTION

The growth was quite strong with ring formation in 6 to 7 days at 30° C. The clouding in the medium was very persistent and the amount of sediment considerable. In some cases the ring extended out over the surface of the medium to some extent, but not sufficiently to be termed a pellicle. This ring was very easily broken up and most of it settled to the bottom of the tube. In six weeks time the white precipitate in the tubes was 10 to 12 m.m. deep. The tubes at 37° C. became almost entirely clear in 12 days, only a moderate amount of sediment remaining.

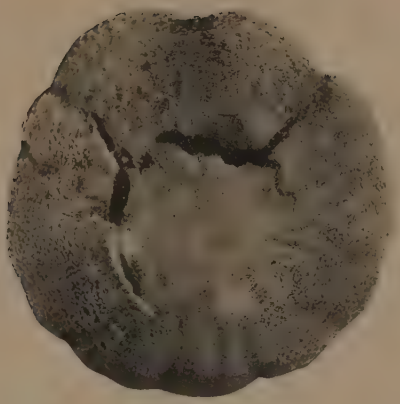

FIGURE 1. Montreal melon showing infection in a natural crack.

FIGURE 2. Montreal melon showing early stage of rot, at spot just at right of center, 24 hours after needle prick inoculation with a pure culture of *Bacillus melonis*.

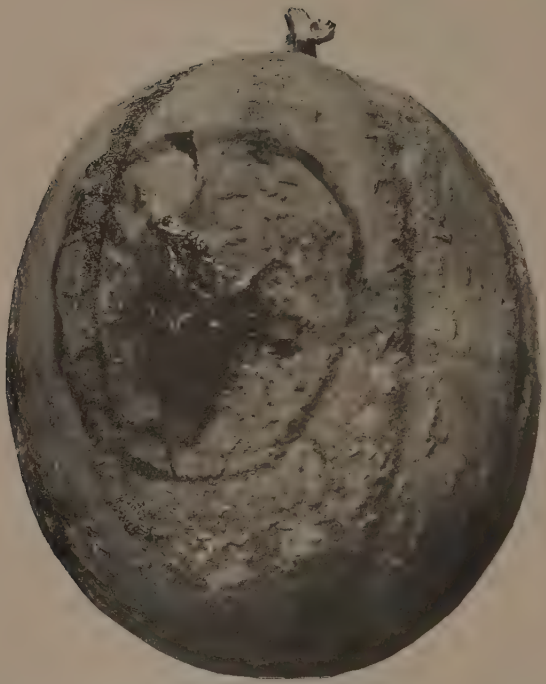

FIGURE 3. Muskmelon showing progress of the rot 64 hours after needle prick inoculation with pure culture of *B. melonis*.

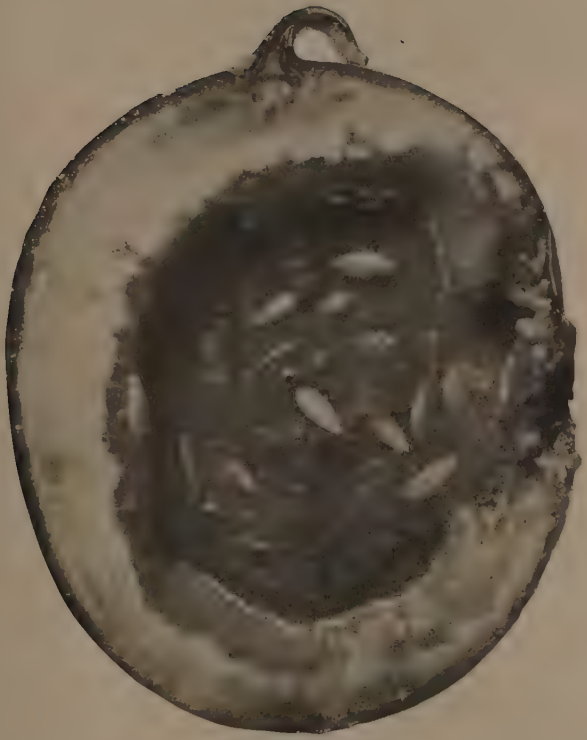

FIGURE 4. Showing same melon as Figure 3, cut open, lengthwise. The flesh was completely rotted on the right side in the neighborhood of the inoculation, the interior cavity was fully invaded and the rot was progressing through the flesh on all sides as shown by the darkened margin.

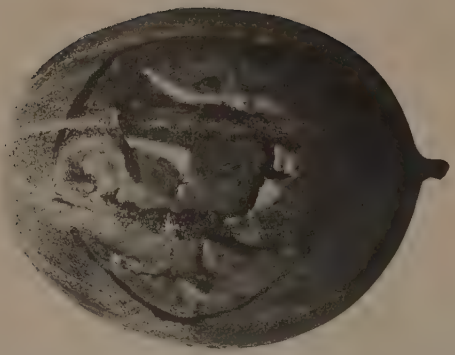

FIGURE 5. Muskmelon 38 hours after needle stab inoculation with pure culture of *B. melonis*. Fully one-third of the visible surface was then rotted, as outlined by the ink line encircling it.

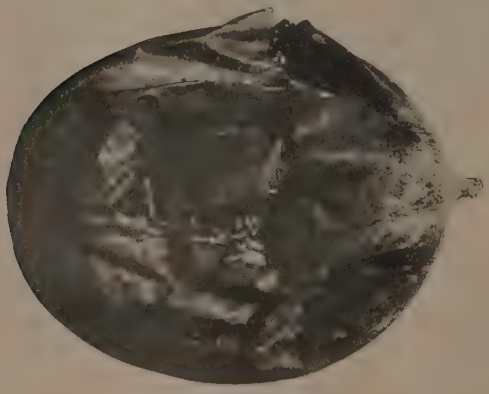

FIGURE 6. Same melon 65 hours after inoculation

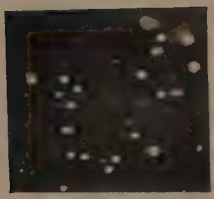

FIGURE 7. Agar plate colonies of *B. melonis*, 3 days growth at 25° C. Natural size.

FIGURE 8. Agar plate colony of same age showing spreading type associated with abundant moisture.

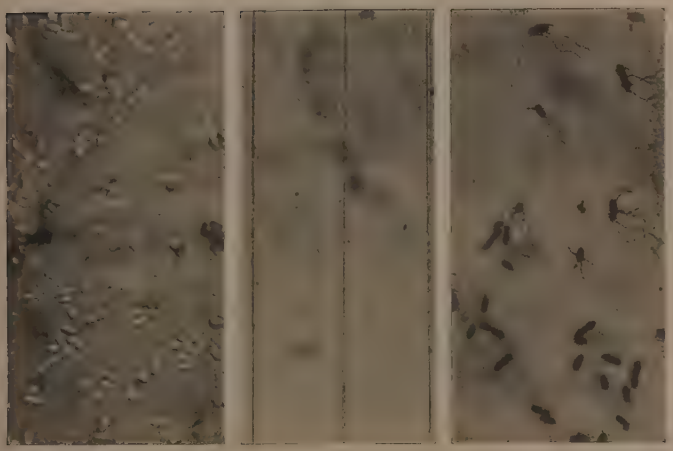

FIGURE 9. Photomicrograph of *B. melonis* showing living organisms in agar hanging block culture, × 1350 (Zeiss 1/12 obj., ocular No. 4).

FIGURE 10. Photomicrograph under same lenses showing 10 micron divisions of stage micrometer.

FIGURE 11. Photomicrograph of *B. melonis* with flagella stained by Löwitz method, × 1350.

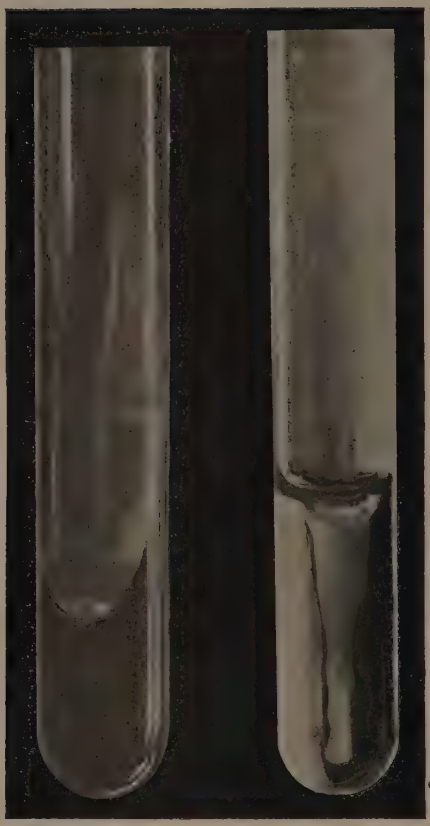

FIGURE 12. Cultures of *B. melonis*. That at left is an agar stroke culture, 3 days old at 30° C. That at right is a gelatin stab culture, 75 hours growth at 20° C.

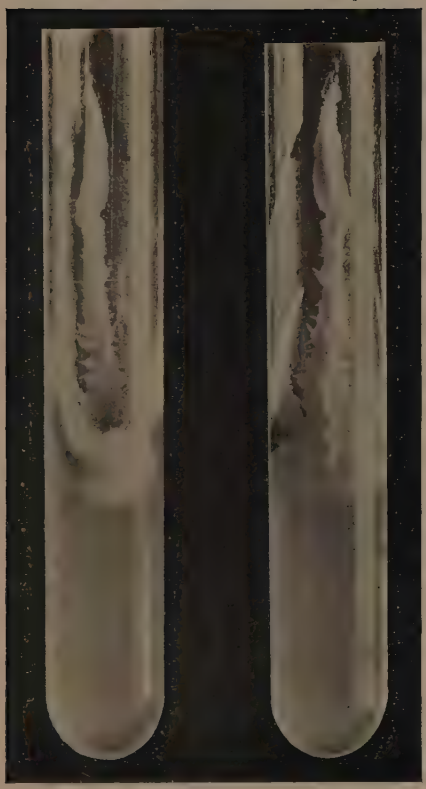

FIGURE 13. Cultures of *B. melonis* on silicate jelly slants, 12 days' growth, (30° C.). The tube at the left shows the face or front view. The tube at the right shows the same seen from the back, i. e., looking through the medium.

FIGURE 14. Fermentation tube culture of *B. melonis* in milk, 13 days old. Note that the closed arm of the tube is fully occupied by gas, except for the column of shrunken curd.

USCHINSKY'S SOLUTION

A good growth occurred with strong clouding in 24 hours. This increased until the end of the second or third day and remained undiminished at the end of 12 to 15 days. There was a pronounced ring and pellicle formation. The pellicle broke up readily upon slight disturbance of the tube, and in a large measure sank to the bottom. In 10 to 15 days this appeared to be somewhat viscid, not breaking up readily upon shaking. The medium was not viscid. There was a disagreeable odor from the tubes suggestive of hydrogen sulphid.[1] The growth in this medium was more pronounced than upon any other fluid medium, unless it was asparagin broth.

COHN'S SOLUTION

Cultures made upon this medium gave no growth.

SPECIAL MEDIA[2]

A. *Whey medium.* Good growth occurred in the closed arm in fermentation tubes within 24 to 48 hours. The clouding increased until the end of the sixth or seventh day and then seemed to decrease. Much sediment formed in the connecting arm. Growth in the open arm was slightly better than in the closed arm in cultures one to two days old.

B. *Curd medium.* Fermentation tubes showed slight clouding in the closed arm 24 hours after inoculation, and fairly well marked clouding in 48 hours. This almost disappeared in 10 days. The curd in the open arm not covered by water was somewhat decomposed by the organism.

C. *Curd medium with lactose.* There was moderate clouding in the closed arm in fermentation tubes at the end of 48 hours.

D. *Casein medium with mannite.* This medium, which had a bluish white appearance when inoculated, at the end of 48

[1]See in this connection page 407.

[2]The composition and methods of making these special media are discussed later in connection with the physical and biochemical characters of the organism on pages 402 and 403.

hours was pure white. The change seemed to be as marked in the closed as in the open arm. It coagulated in four or five days. There was slight separation in the tubes in 7 days, the fluid after separation of the curd remaining clear. The amount of separation, however, was slight even at the end of 15 days.

E. *Casein medium.* No visible change occurred in the fermentation tubes until 10 to 12 days after inoculation. The medium at that time was somewhat whiter than in the checks. It is naturally quite milky so that slight clouding would not have been noticed.

## PHYSICAL AND BIOCHEMICAL CHARACTERS

### TEMPERATURE RELATIONS

*Maximum temperature.* Peptonized beef bouillon having a reaction of +15 was clouded within five hours at 44° C., when inoculated from bouillon cultures. No growth occurred in this medium at 47° C., and after six hours' exposure at this temperature the bouillon was not clouded at room temperature.

*Minimum temperature.* Peptonized beef bouillon having a reaction of +15 was clouded in 14 to 21 days at 1° C. The minimum temperature would, therefore, appear to be about 0° C.

*Growth at different temperatures.* The optimum temperature for growth is about 30° C., but very good growth was secured at laboratory temperature, 22° to 25° C. In all of the work so far as practicable four different temperatures were used. One thermostat was held at 18 to 20°; one at 24° to 25°; one at 30°; and one at 37.5°. The best growth was secured in the 30° thermostat. At 37.5° there was always a slight growth and on some media a fair growth at first, but all clouding due to growth generally disappeared within a few days. At 25° a quite pronounced clouding of broth occurred in 24 hours; at 20° the same amount of clouding was not apparent until after 48 hours.

*Thermal death point.* In determining the thermal death point, transfers were made from broth cultures 1 to 3 days old into tubes of nearly uniform diameter, 14 cm. ×1.5 cm., having a wall about .8 mm. thick and which contained 10 c.c. of nutrient broth, having a reaction of +10 Fuller's scale. These inoculated tubes were allowed to stand a short time, 30 minutes to an hour and a half, before heating. They were then heated in a large water bath by immersing the tubes for at least four-fifths of their length in water, which was held at the desired temperature by a very large calcium chlorid thermo-regulator, and was kept constantly in circulation by a small agitator operated by a motor. This combination of stirrer and thermo-regulator held the desired temperature within 0.1° C. The temperature inside the tubes was noted at intervals of 2 minutes during these exposures. It was found to average about as follows:

| Temperature of water during 10 minutes exposure | Temperature inside tubes after immersion for: 2 minutes | 4 minutes | 6 minutes |
|---|---|---|---|
| 45 | 43.2 | 44.5 | 44.9 |
| 46 | 44.4 | 45.6 | 45.9 |
| 47 | 45.6 | 46.8 | 46.9 |
| 48 | 46.5 | 47.4 | 48.9 |
| 49 | 47.4 | 48.9 | 48.9 |
| 50 | 48.3 | 49.9 | 49.9 |
| 51 | 49.5 | 50.9 | 50.9 |

Two accurate thermometers were used, one being kept in the water bath all the time, while the other was placed in a 10 c.c. tube of the medium used, with the bulb nearly at the bottom of the tube and cotton placed around the thermometer at the top of the tube. In trials conducted in this way, the organism was in all cases killed by ten minutes' heating in the bath at 50° C.; in a few cases the cultures were killed at 49° C. The growth was retarded somewhat at 47° and very decidedly at 49°. Immersion at 45° did not perceptibly retard growth.

### Growth in carbon dioxid

Two tubes of freshly steamed milk, azolitmin milk, agar stab, agar stroke, gelatin stab, broth and Uschinsky's solution were inoculated and placed at once in a Novy jar. Car-

bon dioxid from a Kipp generator was passed through a 10% solution of sodium carbonate, a 10% solution of potassium permanganate, freshly boiled distilled water, and then into the Novy jar. The gas was allowed to pass through the jar rather rapidly for an hour; the jar was then closed and sealed. It remained closed for 12 days. Upon opening no sign of growth was found upon any of the media, except for a slight trace in the gelatin and the agar stabs. The tubes were placed in an incubator at 25° C., and in 24 hours there was good growth in broth, agar stab, agar stroke, and gelatin stab with liquefaction of the gelatin.

### INDOL PRODUCTION

Cultures were made in Dunham's peptone solution and in the 1 percent solution recommended by the American Public Health Association.

Cultures 10 days old gave a slight rose color, indicating indol production. This reaction was somewhat more pronounced at 37° C. than at the other temperatures, and was more pronounced in all the tubes after warming at 70° C. for about five minutes. There was very little difference in the reaction of the two broths, but the indol production in the 1% peptone seemed to be a little stronger than in the other. The tubes were tested at the end of 20 days and well marked indol reaction was secured in the Dunham's solution after the tubes were allowed to stand for a few moments. In the 1% peptone there was a fair reaction upon standing, but not apparently much more than in the 10 day old cultures. The indol test was made by adding to the tubes about 5 to 10 drops of C. P. sulphuric acid, allowing the tubes to stand a few moments, and then adding to each tube 1 c.c. of a .01% solution of sodium nitrite. There was no indication that nitrites were produced since the reaction was secured only when the sodium nitrite was added. Check tubes of the solution gave a slight brownish reaction, which was quite different from the rose red reaction secured in the cultures.

### NITRITE PRODUCTION

Nitrates were reduced to nitrites upon each of the following media: (A) 1 liter of water, 2.5 grams Liebig's extract of beef, 3 grams C. P. potassium nitrate, 10 grams Witte's peptone. (B) 1 liter of distilled water, 1 gram Witte's peptone, and 1 gram C. P. potassium nitrite. (American Public Health Association). The presence of nitrites was determined by the starch iodine method. Cultures of A 5 days old showed strong nitrite production, while cultures of B at the same age showed very slight bluish discoloration, after standing 15 to 20 minutes. Tests were also made at the end of 10 days, at which time the tubes of B incubated at 37° gave a fairly well marked reaction within 2 to 3 minutes. Tubes of B incubated at other temperatures showed a more pronounced reaction than the 5 day old cultures, but it was quite slow in appearing. Tubes of A gave as strongly marked reaction at the end of five days as at the end of 10 days.

### AMMONIA PRODUCTION

The ammonia production was determined by distillation of the culture, or of the uninoculated material, in a liter flask with heavy magnesium oxid, collecting the distillate in a small flask containing a known amount N/20 hydrochloric acid. The distillation was continued for about 30 minutes and the distillate titred with N/20 hydrochloric acid or sodium hydroxid as was required, using cochineal as an indicator.

Cultures were made in 500 c.c flasks containing, respectively, 100 c.c, of nutrient broth, nutrient gelatin or milk. When these cultures were 10 days old they were distilled and the ammonia in the distillate determined. Control distillations were also made on 100 c.c. samples, both of uninoculated milk and broth. The results secured showed that there was no ammonia production in any of the above mentioned media, since the amount of ammonia secured from the checks was as large as the amount secured from the cultures.

Distillation was also made of broth from fermentation tube cultures of urea and asparagin. From 100 c.c. of asparagin broth, taken from fermentation tubes 2 days after inoculation, sufficient ammonia was secured by distillation to neutralize 5.25 c.c. of N/1 hydrochloric acid. The same amount of broth from the cultures 4 days old required 16.3 c.c. of N/1 hydrochloric acid to neutralize the distillate. Thirty-five c.c. of uninoculated asparagin broth required about 2.8 c.c. of N/20 hydrochloric acid to neutralize the distillate. The amount required for the uninoculated asparagin broth was about the same as that for uninoculated nutrient broth, showing that the only ammonia secured from the check distillation of asparagin broth was what would have come from the nutrient broth itself. The organism evidently broke down the asparagin, forming large quantities of ammonia in so doing.

Material from fermentation tube cultures of urea was also distilled. One hundred c.c. of urea broth from 2 days old cultures required 3.9 c.c. of N/1 hydrochloric acid, 4 days old cultures required 3.8 c.c., 15 days old cultures required 4 c.c. and 40 c.c. of uninoculated broth required 1.6 c.c. From this it is evident that ammonia was secured from the urea as well as from the nutrient broth in the uninoculated material, and that there was no ammonia produced by the organism through breaking down of the urea. There was also evidence of slight alkali production in fermentation tubes of maltose 18 days after inoculation, however, as will be noted in the table showing titrations of that broth. No distillations were made.

### ACID PRODUCTION

Acid production was determined upon milk, litmus milk, and various fermentation broths. The latter were made by adding to nutrient broth 2% of either maltose, lactose, saccharose, dextrose, glycerin, urea, asparagin or mannite. The acid production in milk was far more pronounced than in any of the other media

tested. Litmus milk was reddened in 2 days. The greatest amounts of acid were produced in the fermentation broths carrying saccharose, lactose and dextrose. Tubes of maltose showed very slight acid production at the end of 4 days, and at the end of 18 days showed slight alkali production. There is some question whether the large amount of acid in milk arises from the fermentation of the lactose, or of some other compound found in the milk. A more detailed discussion of this milk fermentation will be found under the head of gas production. A tabular summary of the acid production on milk and upon various fermentation broths is given on pages 396-399. The results secured with asparagin broth are omitted, since a large number of titrations, both from inoculated and control tubes, indicated that a chemical reaction occurred between the asparagin and the sodium hydroxid making the neutralization point somewhat obscure. Titrations were made with N/20 sodium hydroxid, using phenolphthalein as an indicator. Five c.c. samples were taken from each tube of the fermentation broths. The entire quantity of milk, 10 c.c., was used in the case of these cultures, and the tubes were carefully rinsed with some of the water used in making the dilutions. The sample in each case was diluted with 45 c.c. of distilled water and was boiled 2 to 4 minutes to drive off carbon dioxid.

### EXPLANATION OF TABLES

The first column gives age of the culture when titred; the second column, the temperatures at which the cultures were incubated; the double column headed "First series" gives the reactions expressed in degrees of acidity on Fuller's scale of one set of cultures, duplicate cultures being titred in most cases; the double column headed "Second series" gives the reactions, similarly expressed, of another set of cultures; and the single column to the right gives the average reaction for all tubes at any given age and temperature, and the average reaction of all control tubes.

ACID PRODUCTION IN MILK

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 17.2 | 18.5 | 12.9 | 13.7 | 15.6 |
| | 25 " | 19.3 | 21.2 | 14.5 | 13.2 | 17.6 |
| | 30 " | 19.9** | 21.7** | 17.1** | 18.0** | 19.2 |
| | 37 " | 19.6 | 18.0 | 10.7 | 11.2 | 14.9 |
| 2 days | 20 " | 18.5 | 19.1 | 13.8 | 14.4 | 16.5 |
| | 25 " | 18.9 | 20.5* | 16.4* | 16.6* | 18.1 |
| | 30 " | 32.3* | 20.2* | 19.6* | 20.2* | 20.6 |
| | 37 " | 18.8 | 19.3 | 12.1 | 12.1 | 15.6 |
| 4 days | 20 " | 18.3* | 18.9* | 17.0* | 16.6* | 17.7 |
| | 25 " | 18.4* | 20.6* | 23.5* | 16.8* | 19.8 |
| | 30 " | 23.0* | 25.3* | 26.6* | 27.1* | 25.5 |
| | 37 " | 23.5 | 24.3 | 16.0 | 12.7* | 19.1 |
| 10 days | 20 " | 21.9* | 21.5* | 19.7* | 15.9* | 19.8 |
| | 25 " | 38.6* | 41.4* | 36.0* | 34.1* | 37.5 |
| | 30 " | 42.5* | 46.6* | 46.4* | 46.5* | 45.5 |
| | 37 " | 26.8 | 27.8 | 31.2* | 21.5 | 26.8 |
| 21 days | 20 " | 53.8* | 48.3* | 47.0* | 49.5* | 49.7 |
| | 25 " | 57.3* | 58.2* | 59.0* | 62.2* | 59.2 |
| | 30 " | 54.2* | 45.9* | 52.8* | 54.5* | 51.9 |
| | 37 " | 23.8 | 25.4 | 29.0* | 34.6* | 28.2 |
| | Controls | 15.4 | 15.1 | 10.1 | 10.1 | 12.9 |
| | | 15.7 | 14.6 | 10.6 | 11.5 | .... |
| | | 15.5 | 16.1 | 10.6 | 9.5 | .... |
| | | 14.6 | 15.4 | 11.7 | 10.4 | .... |
| | | 15.5 | 15.1 | 10.5 | 10.8 | .... |

A single star following the figures for any tube indicates that the milk was entirely coagulated. Double stars indicate that portions of the milk had so coagulated that a lump was noticed when it was poured out.

ACID PRODUCTION IN MALTOSE

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 15.0 | 15.2 | 16.7 | .... | 15.6 |
| | 25 " | 20.3 | 20.3 | 14.9 | 14.4 | 17.5 |
| | 30 " | 21.9 | 21.1 | 16.6 | 17.2 | 19.2 |
| | 37 " | 15.9 | 15.7 | 12.7 | .... | 14.8 |
| 2 days | 20 " | 18.0 | 17.5 | 12.7 | .... | 16.1 |
| | 25 " | 13.1 | 14.7 | 12.5 | 13.5 | 13.5 |
| | 30 " | 13.7 | 15.9 | 11.4 | 11.8 | 13.2 |
| | 37 " | 14.1 | 14.8 | 11.1 | .... | 13.3 |
| 4 days | 20 " | 14.3 | 12.8 | 14.2 | .... | 13.8 |
| | 25 " | 12.7 | 11.5 | 14.7 | 12.2 | 12.8 |
| | 30 " | 12.9 | 14.1 | 18.4 | 16.6 | 15.5 |
| | 37 " | 16.0 | 16.6 | 20.0 | .... | 17.5 |
| 18 days | 20 " | .... | .... | 7.3 | .... | 7.3 |
| | 25 " | .... | .... | 6.7 | 9.5 | 8.1 |
| | 30 " | .... | .... | 4.7 | 2.7 | 3.7 |
| | 37 " | .... | .... | 34.8 | .... | 34.8 |
| | Controls | 14.2 | 12.6 | 10.2 | 12.0 | 12.3 |

ACID PRODUCTION IN SACCHAROSE

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 14.0 | 13.1 | 13.4 | .... | 13.5 |
| | 25 " | 18.0 | 17.5 | 13.5 | 15.0 | 16.0 |
| | 30 " | 19.6 | 20.0 | 15.2 | 15.2 | 17.5 |
| | 37 " | 14.9 | 12.4 | 10.1 | .... | 12.5 |
| 2 days | 20 " | 19.1 | 17.9 | 14.0 | .... | 17.0 |
| | 25 " | 20.7 | 20.1 | 17.5 | 15.7 | 18.5 |
| | 30 " | 20.4 | 20.2 | 17.4 | 18.0 | 19.0 |
| | 37 " | 11.5 | 10.9 | 13.7 | .... | 12.0 |
| 4 days | 20 " | 21.1 | 20.8 | 17.8 | .... | 19.9 |
| | 25 " | 21.1 | 20.6 | 18.1 | 17.7 | 19.4 |
| | 30 " | 21.9 | 22.7 | 19.5 | 21.2 | 21.3 |
| | 37 " | 14.6 | 14.3 | 23.0 | .... | 17.3 |
| 18 days | 20 " | .... | .... | 18.0 | .... | 18.0 |
| | 25 " | .... | .... | 19.6 | .... | 19.6 |
| | 30 " | .... | .... | 20.5 | 21.4 | 21.0 |
| | 37 " | .... | .... | 27.5 | .... | 27.5 |
| | Controls | 11.7 | 11.3 | 8.5 | 10.0 | 10.4 |

ACID PRODUCTION IN LACTOSE

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 10.4 | 10.4 | 8.4 | .... | 9.7 |
| | 25 " | 18.6 | 12.4 | 11.1 | 11.8 | 13.5 |
| | 30 " | 18.8 | 17.6 | 13.4 | 12.1 | 15.5 |
| | 37 " | 12.6 | 12.3 | 8.9 | .... | 11.3 |
| 2 days | 20 " | 14.5 | 13.5 | 12.8 | .... | 13.6 |
| | 25 " | 18.1 | 18.1 | 13.8 | 14.0 | 16.0 |
| | 30 " | 22.2 | 23.5 | 13.5 | 13.8 | 18.3 |
| | 37 " | 11.0 | 10.4 | 11.8 | .... | 11.1 |
| 4 days | 20 " | 18.6 | 19.7 | 14.8 | .... | 17.7 |
| | 25 " | 22.0 | 20.0 | 14.5 | 15.3 | 18.0 |
| | 30 " | 19.2 | 18.1 | 16.8 | 17.6 | 17.9 |
| | 37 " | 13.9 | 14.0 | 20.5 | .... | 16.1 |
| 18 days | 20 " | .... | .... | 14.7 | .... | 14.7 |
| | 25 " | .... | .... | 17.8 | 17.0 | 17.4 |
| | 30 " | .... | .... | 19.5 | 18.8 | 19.2 |
| | 37 " | .... | .... | 22.8 | .... | 22.8 |
| | Controls | 10.6 | 11.0 | 10.0 | 9.0 | 10.2 |

ACID PRODUCTION IN DEXTROSE

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 10.4 | 11.0 | 14.8 | .... | 12.1 |
| | 25 " | 18.1 | 17.5 | 15.7 | 13.9 | 16.3 |
| | 30 " | 19.0 | 19.1 | 16.2 | 14.8 | 17.3 |
| | 37 " | 14.9 | 14.4 | 12.0 | .... | 13.8 |
| 2 days | 20 " | 14.4 | 13.7 | 13.4 | .... | 13.8 |
| | 25 " | 16.2 | 19.1 | 14.9 | 13.6 | 16.0 |
| | 30 " | 22.0 | 21.5 | 17.5 | 18.4 | 19.9 |
| | 37 " | 11.7 | 11.9 | 16.0 | .... | 13.2 |
| 4 days | 20 " | 17.0 | 18.8 | 17.8 | .... | 17.9 |
| | 25 " | 17.8 | 23.0 | 18.8 | 19.2 | 19.7 |
| | 30 " | 24.6 | 25.1 | 20.8 | 22.3 | 23.2 |
| | 37 " | 13.6 | 13.4 | 29.5 | .... | 18.8 |
| 18 days | 20 " | .... | .... | 13.7 | .... | 13.7 |
| | 25 " | .... | .... | 19.9 | 17.5 | 18.7 |
| | 30 " | .... | .... | 21.5 | 19.6 | 20.6 |
| | 37 " | .... | .... | 29.4 | .... | 29.4 |
| | Controls | 10.9 | 11.3 | 10.0 | 10.0 | 10.6 |

ACID PRODUCTION IN GLYCERIN

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 9.2 | 10.2 | 8.2 | .... | 9.2 |
| | 25 " | 9.7 | 9.6 | 8.8 | 8.5 | 9.1 |
| | 30 " | 10.9 | 11.0 | 8.7 | 8.8 | 9.9 |
| | 37 " | 13.0 | 12.1 | 10.0 | .... | 11.7 |
| 2 days | 20 " | 10.0 | 10.4 | 8.5 | .... | 9.6 |
| | 25 " | 10.6 | 11.7 | 10.3 | 10.3 | 10.7 |
| | 30 " | 11.3 | 11.0 | 9.0 | 9.5 | 10.2 |
| | 37 " | 11.3 | 10.7 | 11.3 | .... | 11.1 |
| 4 days | 20 " | 11.0 | 10.4 | 8.3 | .... | 9.9 |
| | 25 " | 11.3 | 11.6 | 11.0 | 13.0 | 11.7 |
| | 30 " | 12.0 | 11.8 | 10.2 | 10.9 | 11.2 |
| | 37 " | 13.2 | 12.9 | 10.0 | .... | 12.0 |
| 18 days | 20 " | 16.1 | 14.0 | .... | .... | 15.1 |
| | 25 " | 16.6 | .... | .... | .... | 16.6 |
| | 30 " | 15.1 | 15.6 | .... | .... | 15.4 |
| | 37 " | 19.2 | 18.0 | .... | .... | 18.6 |
| | Controls | 9.5 | 10.0 | 8.1 | 8.2 | 9.0 |

ACID PRODUCTION IN MANNITE

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 14.4 | 13.4 | 10.2 | .... | 12.7 |
| | 25 " | 16.6 | 14.8 | 13.4 | 13.0 | 14.5 |
| | 30 " | 17.0 | 16.6 | 14.0 | 14.4 | 15.5 |
| | 37 " | 10.4 | 10.8 | 14.3 | .... | 11.8 |
| 2 days | 20 " | 16.6 | 17.2 | 13.5 | .... | 15.8 |
| | 25 " | 15.9 | 17.0 | 13.8 | 13.6 | 15.1 |
| | 30 " | 15.4 | 17.3 | 13.2 | 13.2 | 14.8 |
| | 37 " | 20.6 | 19.9 | 16.5 | .... | 19.0 |
| 4 days | 20 " | 15.3 | 16.9 | 15.2 | .... | 15.9 |
| | 25 " | 15.1 | 16.2 | 16.2 | 18.0 | 16.4 |
| | 30 " | 16.4 | 18.4 | 17.2 | 15.7 | 16.9 |
| | 37 " | 22.6 | 21.1 | 16.8 | .... | 20.2 |
| 15 days | 30 " | 16.2 | 17.0 | .... | .... | 16.6 |
| | 25 " | 17.5 | 18.0 | .... | .... | 17.8 |
| | 30 " | 20.0 | 21.2 | .... | .... | 20.6 |
| | 37 " | 25.0 | 25.2 | .... | .... | 25.1 |
| | Controls | 9.2 | 9.6 | 8.0 | 8.5 | 8.8 |

ACID PRODUCTION IN UREA

| Age | Temperature | Acidity expressed in degrees of Fuller's scale | | | | |
|---|---|---|---|---|---|---|
| | | First series | | Second series | | Average |
| 1 day | 20°C. | 15.9 | 16.2 | 10.0 | .... | 14.0 |
| | 25 " | 11.4 | 10.7 | 10.7 | 10.3 | 10.8 |
| | 30 " | 8.7 | 8.2 | 10.4 | 9.1 | 9.1 |
| | 37 " | 12.8 | 11.7 | 10.0 | .... | 11.5 |
| 2 days | 20 " | 11.8 | 11.2 | 8.6 | .... | 10.5 |
| | 25 " | 9.0 | 10.3 | 8.7 | 8.8 | 9.2 |
| | 30 " | 12.6 | 11.6 | 9.7 | 10.5 | 11.1 |
| | 37 " | 11.2 | 9.2 | 9.0 | .... | 9.8 |
| 4 days | 20 " | 10.8 | 9.7 | 9.5 | .... | 10.0 |
| | 25 " | 9.8 | 7.9 | 11.3 | 11.7 | 10.2 |
| | 30 " | 12.6 | 13.1 | 9.4 | 9.5 | 11.1 |
| | 37 " | 9.0 | 10.4 | 9.8 | .... | 9.7 |
| 15 days | 20 " | 12.0 | 11.5 | .... | .... | 11.8 |
| | 25 " | 12.4 | .... | .... | .... | 12.4 |
| | 30 " | 12.3 | 13.3 | .... | .... | 12.8 |
| | 37 " | 13.8 | 16.3 | .... | .... | 15.1 |
| | Controls | 9.8 | 10.0 | 9.8 | 9.2 | 9.7 |

GAS PRODUCTION

Cultures were made in fermentation tubes of nutrient broth containing 2% additions, respectively, of lactose, maltose, dextrose, saccharose, glycerin, asparagin, urea, and mannite. In order to secure convincing data the entire series was run three times, except urea, which was run only twice. In each trial, a total of 8 tubes of each medium were used, two tubes being carried at each of the four temperatures, 20°, 25°, 30°, 37° C. There was no sign of gas production in any of these tubes, except in two out of six of the asparagin tubes at 30° C. and one out of six of the asparagin tubes at 25°. Two of these three asparagin tubes produced only 5% gas, and the other tube only 8%. This gas was tested as carefully as possible without special apparatus, and found to be about half carbon dioxid. The residue was thought to be nitrogen, since no explosion or ignition could be secured.

*Comparison with B. coli.* For purposes of comparison inoculations of *Bacillus coli,* taken as a type of actively fermentative bacteria, were made into fermentation tubes containing nutrient broth and 2% additions of saccharose, dextrose, lactose, maltose, glycerin, asparagin or mannite. Gas production occurred in these cultures of *B. coli* amounting to about 70% in mannite, 28% in maltose, 28% in dextrose, 25% in lactose, 6% in glycerin. No gas production appeared in asparagin or saccharose.

Shaken cultures were also made in agars containing either 2% or 5% of lactose, saccharose, dextrose, glycerin, mannite or plain nutrient agar. No gas production appeared in any of these cultures. Cultures of *B. coli* were made upon each of the above media at the same time, and abundant gas production occurred in all such, save those upon plain nutrient agar.

*Gas from milk.* When the preliminary cultural characters were observed in the fall of 1907, it was noted that gas was produced in tubes of milk and litmus milk after they were a week to 10 days old, and that none was secured in nutrient agar contain-

ing lactose, saccharose, or dextrose. In the course of the final studies it was further noted that no gas was produced in fermentation tubes of nutrient broth containing these various carbohydrates. The gas produced in tubes of milk was quite noticeable, especially if the tubes remained undisturbed for 3 or 4 days after they were about a week old. In such cases, if the tube was gently rapped, many small and some large bubbles would rise to the top of the whey. This phenomenon seemed so strange that fermentation tubes of milk were prepared, sterilized, and inoculated in the same manner as ordinary tubes of milk. In the course of 6 to 15 days a small amount of gas gathered in the top of the closed arm of all such tubes. After this gas production began it proceeded quite rapidly, completely filling the closed arm in 4 to 6 days. In many cases it was noted, after the closed arm filled, bubbles of gas continued to escape from it through the open arm. These trials with fermentation tubes of milk have been repeated several times, including, all told, some 75 tubes, and like results were secured in every case. The gas produced was tested by shaking with a 2% solution of sodium hydroxid and found to be more than 99% carbon dioxid. The small portion of gas not absorbed was tested as carefully as might be and appeared to be nitrogen. Owing to the difficulty of testing so small a bubble, the method followed in the earlier trials was to collect the small bubbles from 3 or 4 tubes into one small tube, the transfer being made under water. This gas thus collected in the one tube was then carefully brought into contact with an open flame by forcing it slowly out of one end of the tube, which was held in the edge of the gas flame. No explosion or ignition took place. An eudiometer tube was secured for use in later trials, and the small bubbles from two tubes were collected over mercury. The pressure on this gas was then bought up to normal by the ordinary levelling device, the amount noted, oxygen in excess of the amount required to form water added, atmospheric pressure again restored, and the total amount again noted. The mixture

of gases was then sparked, but no explosion or diminution in volume took place. A quantity of hydrogen, slightly greater than the amount of the unknown gas, was added, and the mixture of gases again sparked. This time an explosion ensued, and the volume diminished proportionately to the water value of the added hydrogen. Repetition afforded exactly similar results. It was therefore concluded that the small bubble remaining in the fermentation tubes after the absorption of carbon dioxid was not hydrogen.

*Special media.* The amount and composition of the gas produced from milk was so striking that the following special media were made up in order to see if further light might be secured.

A. *Whey medium.* Freshly centrifuged milk, amphoteric to neutral litmus, was secured, and to 200 c.c. was added .01 c.c. of rennet. This was allowed to stand at a temperature of about 40° C. for 2.5 hours. At the end of an hour the milk was coagulated, and at the end of 2.5 hours the whey and curd were partially separated. The milk was then placed in an Arnold sterilizer and heated at 99° C. for 10 minutes. This seemed to make the coagulum firmer and force out more of the whey. The latter was then carefully poured out on a filter, as much being secured from the curd as was practicable without squeezing it or removing it from the flask. This filtered whey was then put up in fermentation tubes and sterilized by flowing steam. Inoculated tubes of this medium showed gas production in six to seven days at 25° C. The amount of gas produced was from 30% to 54% of the closed arm, and its analysis afforded results similar to those secured with the gas from milk in that 99% thereof consisted of carbon dioxid.

B. *Curd medium.* The curd remaining after the removal of the whey, used in making the above medium A, was washed thoroughly four times with distilled water by shaking it vigorously and allowing it to stand 10 or 15 minutes before pouring off the

water. The curd was then forced into the closed arm of large fermentation tubes, using distilled water to aid in getting it into its location, and to occupy the larger portion of the space. This medium was sterilized in a steamer, inoculated and incubated in the same manner as with A. At the end of 15 days there was no sign of gas production in any of the tubes.

C. *Curd medium with lactose.* Sufficient lactose was added to the distilled water in two of the fermentation tubes containing curd to make a 4% solution. Inoculated tubes of this medium showed a small bubble of gas in the closed arm at the end of 4 days. This amount had not increased at the end of 15 days, and was considered too small to be tested with any apparatus available at that time.

D. *Casein medium with mannite.* In making this medium 100 c.c. of a saturated solution of calcium hydroxid was used as a solvent for 3 grams of Eimer and Amend's C. P. casein. This stood for about eight hours with frequent stirring, then .05 grams di-potassium acid phosphate ($K^2HPO^4$) was added. The mixture was stirred and allowed to stand over night. A small amount of casein did not go into solution, so it was warmed, filtered, and 2 grams C. P. mannite added to the filtrate, which was then put into fermentation tubes and sterilized by flowing steam. The growth in this medium was very good as may be noted under cultural characters, but there was no sign of gas production in any of the tubes at the end of 30 days.

E. *Casein medium.* This medium was similar to D and made up in the same manner, except that commercial casein, precipitated by sulphuric acid, was used and that no mannite was added. A considerably smaller portion of this casein appeared to go into the solution than of the C. P. casein, but the solution had a slightly more milky appearance than did the other. There was no sign of gas production in any of these tubes.

*Milk analyses.* It was suggested that gas might be produced in the closed arm in fermentation tubes in the absence of

oxygen. In order to test this idea, fermentation tubes of freshly centrifuged milk reacting +12 on Fuller's scale were inoculated, and placed in an atmosphere of carbon dioxid. Some of these tubes were inoculated five days, and some one day previous to placing in a Novy jar, while others were freshly steamed and, as soon as cool, inoculated and placed at once in a Novy jar. These tubes were the ones mentioned in the earlier discussion of growth in carbon dioxid (page 391). No gas had been produced in any of these tubes when the jar was opened at the end of 17 days. The cultures were then placed in an incubator at 25° C. and gas was formed in all the tubes by the end of seven days. The results attained in these trials would indicate that the gas produced in the fermentation tubes of milk was not due to the breaking down of the casein by the organism. With the hope of securing further information along this line determinations were made of the protein, lactose and acidity contents of samples of the culture medium. A quantity of fresh, centrifuged milk was secured and placed in 200 c.c. portions into 300 c.c. Erlenmeyer flasks. An analysis of a single sample was made at once, and the other portions were sterilized by flowing steam at 99° C. for fifteen minutes on each of three consecutive days. One-half of the flasks were then inoculated by adding to each with a sterile pipette 1 c.c. of a 24 to 48 hour broth culture. The inoculated and control flasks were then incubated at 25° C., and one of each taken out for analysis every third day up to and including 15 days after inoculation.

The results of these analyses are given in the following table:

| Age of sample | Control samples | | | Inoculated samples | | |
|---|---|---|---|---|---|---|
| | Protein | Milk sugar | Acidity | Protein | Milk sugar | Acidity |
| Fresh milk | 3.81% | 4.40% | 0.155% | .... | .... | .... |
| 3 days | 3.83 | 4.30 | 0.16 | 3.97% | 4.20% | 0.16% |
| 6 days | 3.90 | 4.15 | 0.17 | 3.77 | 3.25 | 0.18 |
| 9 days | 3.73 | 3.70 | 0.16 | 3.93 | 2.40 | 0.18 |
| 12 days | 3.78 | 3.60 | 0.17 | 3.80 | 2.09 | 0.18 |
| 15 days | 3.93 | 3.40 | 0.17 | 4.02 | 0.69 | 0.44 |

The only thing about the control samples which was thought worthy of special note was the continual diminution of lactose content as age increased. There was practically no change in acidity to account for such diminution and no attempt at explanation will be made here.

The lactose content of the inoculated samples shows a remarkable decrease of 3.51% in twelve days. By deducting from this amount the total diminution of the lactose, 0.90%, noted in the control tubes during the same period, there remained for the inoculated samples a loss of 2.61%, which may be considered as directly due to the activities of this organism. The total gain in acidity of the inoculated samples during the twelve days was 0.28%. Assuming that the organism produced but one molecule of lactic acid from each molecule of lactose, the loss in lactose, 2.61%, was greatly in excess of the amount which would be required to account for this gain in acidity.

The gas produced in the inoculation flasks was very slight on the ninth day, but was quite pronounced on the twelfth day, and appeared to be fully as strong on the fifteenth day.

These results, showing practically no change in the protein content and an excessive loss in the lactose content, with the greater portion of this loss taking place when abundant gas production is occurring, would indicate that the carbon dioxid is formed as a result of fermentation of the milk sugar.

### Alcohol production

It has been suggested that this organism produced a yeast-like fermentation of lactose. All of the work completed up to this point would indicate that such was the case. As alcohol is one of the products of yeast fermentation of sugar, an endeavor was made to determine whether or not alcohol was produced in milk fermented by this bacillus. For this purpose 500 c.c. of fresh centrifuged milk was placed in a 750 c.c. Jena flask and sterilized by flowing steam at 99° for fifteen minutes on each of three consecutive days. This milk was then

inoculated with 3 c.c. of a 24-48 hour broth culture, and left at room temperature, 20-20°. At the end of fifteen days the culture was poured into a larger flask and distilled until about 50 c.c. of distillate was secured. A portion of this distillate was tested as follows: Four or five drops of a 10% solution of potassium hydroxid was added, the mixture warmed to about 50° C., and a solution of potassium iodide saturated with free iodine added, a drop at a time, until the liquid assumed a permanent yellowish brown color. It was then carefully decolorized by adding, drop by drop, more of the potassium hydroxid solution, and set aside. The fluid gave off a distinct odor of iodoform and in a few moments, there was an abundant deposit of yellow crystals in the bottom of the tube. The results of this test would indicate the presence of an alcohol, acetone, or aldehyde in the distillate. This test was repeated with a second portion of the filtrate, and similar results secured, although in this case the formation of several iodoform crystals was noted before the mixture was entirely cleared by the addition of potassium hydroxid. In the absence of a good direct test for so small an amount of alcohol, the following tests, as outlined in Allen's Commercial Organic Analysis, vol 1, were carefully made for the presence of aldehydes: The resinous deposit with sodium hydroxid, the silver mirror with ammoniacal silver nitrate, and the color reaction with excess of phenol-sulphonic acid. Negative results were secured in every case, indicating that there was no aldehyde present. These determinations seemed to warrant the statement that the fermentation of milk caused by this organism results in the production of alcohol.

A careful search has served to reveal but a single instance[1] of a bacterial fermentation of milk which resembles the one above recorded. No analysis of the gas was made in this case, but the author states that the greater part thereof was carbon dioxid.

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[1]Leichmann, Dr. G., "Ueber die freiwillige Sauerung der Milch." Centbl. f, Bakt. Parasit, u. Inkektionskr. 2. Abt., II: 777 (1896).

Mandel[1] mentions a fermentation of lactose which results at the outset in the production of galactose and dextrose which sugars may then undergo alcoholic fermentation. The initial step of this fermentation is spoken of as taking place quite slowly and, since the gaseous fermentation of milk by this organism was quite late in appearing, it was thought that the milk fermentation which it produced might be of such character. The time available for this work did not permit of the testing of a galactose broth, but some interesting results might be secured from such trials.

### HYDROGEN SULPHID PRODUCTION

In determining the production of this gas, strips of absorbent paper moistened with a solution of lead acetate were placed in the tubes, so that the end of the strip hung down nearly to the top of the culture. These strips were remoistened daily with distilled water. The end of the strip inserted in the culture tubes of nutrient broth was decidedly browned, while those hung over the gelatin and cooked potato cultures were slightly tinged. Strips of absorbent paper moistened in the same way were kept in control sterile tubes of the three media used. These gave no indication of browning, showing that the hydrogen sulphid production was caused by the growth of the organism in the culture.

### ODOR

There was no very decided or distinctive odor evolved from any of the media. A fairly strong disagreeable odor arose, however, from cultures on raw turnip, raw carrot and cooked potato. The odor from cultures on cooked potato reminded one of decaying potato tubers in a storage cellar.

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[1]Hammarsten-Mandel, "Text Book of Physiological Chemistry," p. 392, (1902).

GROWTH OVER CHLOROFORM

This medium was prepared by adding .05 c.c. of Squibb's "U. S. P." chloroform to 10 c.c. tubes of sterile bouillon and shaking the tubes slightly. The chloroform was added to the broth with a sterile pipette. Tubes of nutrient broth with such additions of chloroform showed good growth in 24 to 48 hours; the growth was perhaps slightly retarded, but not to any great extent as evidenced by the fact that the clouding was somewhat stronger in the upper part than in the lower part nearer the chloroform.

TOLERATIONS

*Oxalic acid.* Growth was secured in +15 peptonized beef bouillon, acidified to +45 and +47 with oxalic acid. No growth was secured in a similar medium acidified to +52 with oxalic acid.

*Malic acid.* Growth was secured in +15 peptonized beef bouillon, acidified to +37 with malic acid.

*Sodium chlorid.* Bouillon free from sodium chlorid was taken as the basal medium, and the salt was added thereunto to make a series containing, respectively, 1, 2, 3, etc. to 10%. A very good growth ensued in 24 hours in the several media containing from 1 to 6%. A slight growth appeared in 24 hours in that containing 7% and, at the end of 48 hours, a fair growth therein, as well as a very slight growth in that containing 8%. This did not perceptibly increase. No growth having appeared after ten days in the 9 and 10% tubes, transfers were made therefrom and they were found to be sterile.

*Hydrochloric acid.* Ordinary beef bouillon, having a reaction of zero Fuller's scale, was used. Sufficient N/1 hydrochloric acid was added to 100 c.c. of the bouillon to secure reactions of +5, +10, and so on, taking every 5 degrees Fuller's scale up to +50. A very good growth ensued in 24 hours in the medium having a reaction of +20. At the end of 48 hours a good

growth was secured in the medium having a reaction of +20, and very slight growth in that having a reaction of +25. The growth in the +25 medium became slightly stronger at the end of ten days, but otherwise there was no change. No signs of growth appeared in any of the media containing a larger amount of acid.

*Sodium hydroxid.* 100 c.c. portions of ordinary beef bouillon, having a reaction of zero Fuller's scale, were measured out and to each was added the theoretical amount of N/1 sodium hydroxid requisite to secure reactions of —5, —10, and so on for every 5 degrees of Fuller's scale up to —45. Tubes of these media were inoculated and placed in the incubator at 25° C. These showed good growth in 24 hours at 0, fair growth at —5, and slight growth at —10. In 48 hours there was good growth in —5 and —10, fair growth in —15, slight growth in —20. In three days there was good growth in —15, fair growth in —20, slight growth in —25. In five days there was good growth in —20 and —25, fair growth in —30, slight growth in —35. The growth in —35 increased slightly at the end of 20 days, but there was no sign of growth in any of the tubes containing a larger amount of alkali. Sterile control tubes of this broth put up to be —35, —30, and —25, were titrated at the end of 20 days and found to afford a reaction ranging from —14 to —19. This indicated that the sodium hydroxid present when the broth was put up and inoculated had been largely, if not entirely, changed to sodium carbonate. It was, therefore, thought best to repeat the trials keeping the cultures in a carbon dioxid free atmosphere. Tubes of media put up like those above mentioned but ranging in reaction from zero to —30 were inoculated and placed with check tubes in Novy jars. The air was then drawn through, the jars being washed in two bottles of 12% solution potassium hydroxid, a bottle of 1% sulphuric acid, and a bottle of distilled water before reaching the Novy jars. The air was drawn slowly through the jars for about 2 hours, the jars were then carefully sealed, and left at the room temperature 20 to 25°. After 12

days the jars were opened and growth was found in tubes put up to be 0, —5, —10 and —15 Fuller's scale. The growth in the —15 broth was slight and it was only fair in the —10 broth. There was no sign of growth in the —20 broth. Transfers were made from inoculated —15 to —20 broths to tubes of ordinary broth and good growth was secured in tubes from the former, while no sign of growth was obtained in tubes from —20 broth.

Titrations of the control tubes of —10, —15, and —20 broth, made immediately after taking them from the jars, showed them to have reactions of —5, —7.4, and —10.2 respectively. It was, therefore, concluded that the organism was unable to withstand an alkalinity of —10.2 Fuller's scale with sodium hydroxid.

### EFFECT OF GERMICIDES

The only germicides tried were phenol and formalin. These germicides, previously diluted with sterile distilled water, were added to tubes of sterile bouillon, using sterile pipettes.

*Phenol.* Media were used containing .78%, .44%, .18%, .11%, .09%, .08%, .05% and .02% of Merck's "U. S. P." phenol crystals. At the end of 24 hours good growth occurred in the .02% phenol, slight growth in the .05% and the .08% phenols, but no indications of growth in any of the others. In 48 hours fair growth appeared in the .05 and .08% and very slight growth in the .09% and .11%. This growth did not appear to have increased any in 10 days. There was no sign of growth in the media containing respectively, .18%, .44%, and .78% of phenol. After 15 days transfers were made from these tubes to tubes of nutrient broth. Growth was secured in transfers from all inoculated tubes containing .02%, .05%, .08% and .09%, and from three tubes containing .11%. There was no sign of growth from the other two tubes containing .11%, or from any tubes containing a greater amount of phenol.

*Formalin.* Media were used containing .43%, .24%, .10%, .05%, .04%, .02%, .01% and .005% of Bausch and Lomb's

40% formalin. In 24 hours good growth occurred in the .005% and very slight growth in the .01% and .02% trials. In 2 days a fair growth was found in the .01% and slight growth in the .02% and very slight growth in the .04%. There was no increase of growth noted in these tubes in 10 days. There was no signs of growth in media containing .05%, .10%, .24%, or .43%. Transfers from these tubes were made in the same manner as from the phenol tubes. Growth was secured from all tubes containing .005%, .01%, and .02, and from two tubes containing .04%. There was no sign of growth from the other two tubes containing .04%, or from any tubes containing a larger percentage of formalin.

### DESICCATION

Straight needle transfers were made from 24 to 48 hour old broth cultures to sterile cover slips in sterile petri dishes. Care was taken to get as little of the broth as possible from the cover slips, and it was carefully spread out in a very thin film, frequently invisible. These were then allowed to dry down at room temperature, i. e. 20 to 25° C. In order to test the viability of the organisms under such circumstances, six of these covers were transferred with sterile forceps to broth tubes at the end of each 5 minutes, 15 minutes, 30 minutes, 3 hours, 8 hours, 16 hours, 24 hours, 68 hours, and 7 days. Good growth was secured in tubes containing cover slips which had been dried out up to and including 68 hours, but the growth in those dried for 68 hours was quite slow in appearing, showing no sign of growth until 48 hours after transferring the cover slips to the broth. Cover slips dried for 7 days were sterile.

### INSOLATION

Agar plates were poured having a dilution such that the plates had from 200 to 300 colonies. These were allowed to incubate for about a half hour. One-half of each plate was then covered, the plates laid on a tray of snow to prevent overheating,

and then exposed to the direct rays of the sun. These exposures were made between half past one and half past two o'clock, Nov. 21, 1908. A part of the plates were exposed for 15 minutes, others for an hour. These plates were then incubated at 25° C. At the end of 48 hours it was found that the ones exposed for one hour had no colonies on the exposed side and averaged 106 colonies on the covered side. The ones exposed 15 minutes averaged 19 colonies on the exposed side and 68 colonies on the covered side.

## REMEDIAL MEASURES

Detailed studies of this organism, which are given in the previous pages, show that it is capable of development as a wound parasite upon several hosts, but especially upon musk-melons. Its behavior in cultures indicated that it could live indefinitely as a saprophyte under favorable conditions.

No opportunity was offered in the course of these studies to determine experimentally what might be done to check the disease when it was once started in the field. Of course any remedial measures must aim at prevention rather than cure, and must therefore be taken before the disease becomes widespread. Spraying with bordeaux mixture is to be commended as a general preventive of this and related melon diseases. Supporting the melons on stones or otherwise to keep them from contact with the soil, and occasional turning will doubtless give better results than can be secured in any other way. Whenever practicable, irrigation should be practised in a dry time to insure uniform and continuous growth and to avoid cracking of the fruit. Diseased melons in a field should be immediately removed and destroyed, and the crop should be carefully watched for the first appearance of the rot, in order to keep the organisms from the field as much as possible, since the disease may readily be carried from one melon to another by insects. A field in which the rot has been seriously prevalent should not be used the next

year for the growth of melons. An interval of at least three years should intervene between melon crops in such cases. Rotting melons should not be thrown on the compost heap, or be fed to stock, or else, in case this is done, such compost or manure from such animals should not be used on melon fields.

It is probable that little trouble will be experienced during a season which is continuously dry. If, however, the entire season is unusually wet, or if heavy rains follow a dry period, the danger is increased. Under such conditions especial care should be exercised in spraying with bordeaux mixture, and in so supporting and turning the melons as to keep all sides exposed to light and air as much as practicable. The disease, once started in a wet field where no such precautions are taken, spreads very rapidly; and a melon once infected is lost.

## Brief Description of Bacillus Melonis n. sp.[1]

A careful study of the organism described in this paper, and a comparison of its characters with those of other vegetable soft rots, seem to justify its recognition as a distinct species. We are, therefore, proposing for it the name *Bacillus melonis* (new species). The following may be regarded as its most important differential characters:

A bacillus .6-.9 by 1-1.7, actively motile by means of 4-6 peritrichic flagella. Endospores not produced and organisms not stained by Gram's method.

*Nutrient broth.* Strong clouding in 24 hours; no pellicle or ring formation; slight sediment.

*Agar stroke.* Abundant, slightly spreading, contoured, slimy, glistening, translucent, opalescent growth having umbilicate elevation.

*Agar stab.* Filiform growth.

*Agar plate.* Colonies round or amoeboid.

[1]Descriptions based on cultures incubated at 30° C., unless otherwise noted.

*Gelatin stab* at 20° C. Infundibuliform liquefaction in two days.

*Cooked potato.* Growth abundant, spreading and glistening.

*Milk.* Coagulation and some separation in two days; acid production of +55 in twenty-one days; abundant gas production in 10 to 18 days at 25° C.; gas 99% carbon dioxid, the remainder non-explosive with oxygen.

*Fermentation broths.* Good growth in saccharose, lactose, maltose, dextrose, mannite, glycerin, and asparagin; slight growth in urea; good growth in closed arm in mannite, saccharose and asparagin; no growth in closed arm in glycerin and urea; slight alkali production in maltose after 18 days; slight gas production in asparagin in some cases.

*Blood serum.* Slow but distinct liquefaction.

*Fermi's solution.* Strong persistent clouding, ring formation and considerable sediment.

*Uschinsky's solution.* Strong growth; ring and pellicle formation; considerable sediment; odor of hydrogen sulphid.

*Fermi's solution solidified with silicate jelly.* Very abundant growth, slightly spreading and contoured; umbilicate elevation; echinulate edges; slight greenish yellow discoloration of substratum.

*Cohn's solution.* No growth.

*Nitrite production.* Abundant.

*Indol production.* Slight.

*Ammonia production.* Abundant from fermentation tube cultures of asparagin broth. None from nutrient broth, gelatin, milk or urea.

*Toleration of sodium chlorid.* An addition of 7% required to inhibit growth.

*Thermal relations.* Optimum temperature about 30° C.
Maximum temperature about 44.5° C.
Minimum temperature about 0° C.
Death point 49 to 50° C.

*Vegetables rotted.* Muskmelon, citron, carrot, potato, beet, and turnip.

Following the numerical system of recording characters as outlined on the descriptive chart of the Society of American Bacteriologists, endorsed Dec. 31, 1907, the group number of this organism is B. 221,2223032.

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